P-TH-340: Identification Of Immunosuppressive (IFNγ-Stimulated) MSC Morphological Subpopulations Using viSNE, A Tool For Visualizing Cellular Heterogeneity

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Introduction: Although efforts have been made to identify quality attributes of mesenchymal stromal cell (MSC) function, current strategies to characterize MSCs have been unable to effectively address MSC heterogeneity, perhaps leading to inconsistent clinical outcomes. We previously developed techniques to identify population-based morphological features predictive of MSC osteogenic[1] and immunosuppressive capacity[2] and sought to expand upon these methods by quantitatively assessing heterogeneity based on unique morphological subpopulations (SPs). We utilized viSNE (visual stochastic neighbor embedding[3]) to visualize high dimensional morphological data of interferon-gamma (IFNγ) stimulated MSCs to facilitate identification of SPs that predict immunosuppression.

Materials and Methods: MSC cell-lines derived from 13 donors were analyzed for both immunosuppression and morphology using methods outlined in Ref. 2. Morphological data from images of ~$3x10^5$ single cells was uploaded to Cytobank and viSNE was performed on data from multiple experiments. Manual gating of viSNE plots was performed on data from MSCs stimulated with 0, 10, and 50 ng/mL IFNγ. SPs were identified for each IFNγ concentration and both SP frequency and total SP cell numbers were determined for each MSC sample and correlated with their respective immunosuppressive capacity represented as principal component 1 ($PC1^2$) . SPs that significantly correlated with immunosuppression were identified using Bonferroni multiple testing correction.

Results and Discussion: viSNE permitted visualization of high dimensional (20 features) morphological data in two dimensions (Fig 1A), which allowed for identification of distinct SPs using a contour density plot (Fig 1B). Manual gating of SPs allowed for enumeration of each SP for all MSC cell-line/passage populations for multiple experiments. The frequency and total number of cells in each SP was correlated with MSC immunosuppression data. The most significantly correlated SP (SP9) is shown in Fig 1C (exponential model, R=0.83, $p=2x10^{-42}$) and demonstrates that samples with greater immunosuppressive capacity (i.e. lower $PC1^2$) contain greater numbers of SP9 cells. Representative cells from SPs that correlate weakly (SP3, R=0.66) or strongly (SP9, R=0.83) with immunosuppression are shown in Fig 1D and illustrate the ability of viSNE to identify distinct phenotypes.

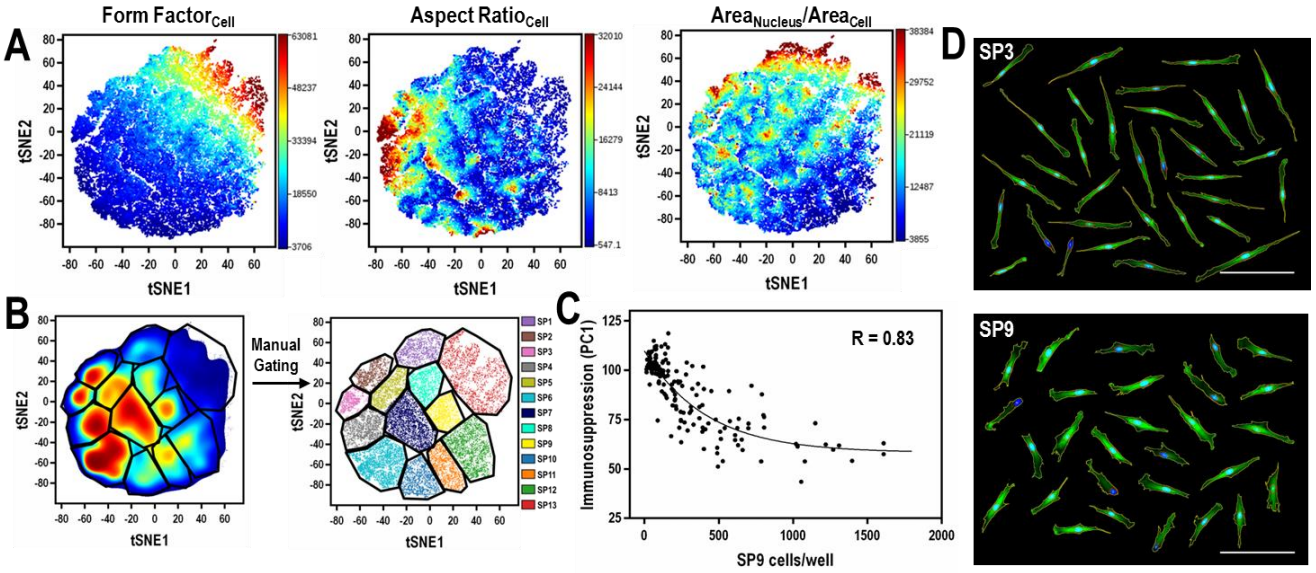

Figure 1. A) viSNE plots color coded by magnitude of individual morphological features. B) Contour density plot representation of data used to identify SPs. C) Positive correlation of total SP9 cells with immunosuppression capacity (as measured by PC1) using exponential decay model (n=160 points). Lower PC1 values indicate greater immunosuppression. D) Representative cells (selected from different samples) illustrating distinct phenotypes (Scale bar = 200 μm).

Conclusions: Using viSNE, we were able to identify morphologically-distinct MSC SPs following IFNγ stimulation, which demonstrated donor and passage-dependent differences in SP frequency and number. We also identified MSC SPs that significantly correlated with MSC immunosuppression for all cell-line/passage groups investigated in this work. This approach could be used as a predictive measure of MSC immunosuppression and could also be used as a tool for identifying molecular markers to enrich for desired functional SPs, as well as optimizing MSC manufacturing conditions to improve MSC functional characteristics.

References: 1) Marklein, RA. *Stem Cells*, 2016:34;935-947. 2) Klinker, MW, Marklein, RA. *PNAS*, 2017:114;2598-2607. 3) Amir, ED. *Nat Biotech*, 2013:31;545-552.